MYCOTAXON

ISSN (print) 0093-4666 (online) 2154-8889 Mycotaxon, Ltd. ©2018

April–June 2018—Volume 133, pp. 229–241

https://doi.org/10.5248/133.229

Tolypocladium dujiaolongae sp. nov. and its allies

Chunru Li[1, 2], Nigel Hywel-Jones[1],
Yupeng Cao[2], Sunghee Nam[3], Zengzhi Li[2*]

[1] *Zhejiang BioAsia Institute of Life Sciences, Pinghu, Zhejiang, China*
[2] *Anhui Provincial Key Laboratory for Microbial Control, Anhui Agricultural University, Hefei 230036 China*
[3] *National Institutes of Agricultural Science & Technology, Department of Agricultural Biology, R.D.A. Suwon, Korea*

* Correspondence to: *zzl@bioasia.com.cn*

Abstract—A common cicada pathogen occurring in bamboo forests of southern China is described as a new species, *Tolypocladium dujiaolongae*. The previously unknown anamorphic state was obtained from discharged ascospores. Based on its morphological characteristics and a molecular analysis of the internal transcribed spacer rDNA sequence, we found that the asexual features of *T. dujiaolongae* are similar to those of *Tolypocladium* species. The typical characteristics of the fungus are described. The fruiting bodies formed on artificial culture media were identical to those of natural specimens, strongly supporting the idea that the isolated strain is the asexual stage of *T. dujiaolongae*. Our results indicate that there is no relationship between *Tolypocladium dujiaolongae* and the famous traditional Chinese medicine *Isaria cicadae*.

Key words—anamorphic–teleomorphic connection, morphology, phylogeny, mycoparasite, taxonomy

Introduction

The use of cordycipoid pathogens of cicadas in Traditional Chinese Medicine (TCM) has a longer documented history (>1500 years) than the TCM usage of *Ophiocordyceps sinensis* (Berk.) G.H. Sung & al. (c. 500 years). The adjective "cordycipoid" refers to all taxa in the families *Clavicipitaceae*, *Cordycipitaceae*, and *Ophiocordycipitaceae*. Use of the cicada pathogen can be traced to a 5th century medical work by Leixiao, titled "Lei's Treatise on Preparing Drugs" (雷公炮炙论), which recorded the preparation of a cicada pathogen, referred to as "cicada flower" (Chan-hua 蝉花) for medical use against childhood disorders. This "cicada flower" has generally been identified as *Isaria cicadae* Miq. (Fig. 1). In the market a small proportion of specimens appear more robust leading to the Chinese folk recognition of a "male (robust) cicada flower" as compared to the "female cicada flower" with its slender synnemata. In southern China, the "male" form is more often called the "single horned dragon" (Dujiaolong, 独角龙) due to its solitary robust stroma.

Shing (1975) reviewed these wild collections and recognised the "male cicada flower" as the teleomorph, which he mistakenly associated with *I. cicadae*. As well as making this link he also distinguished between *Isaria cicadae* and *Cordyceps sobolifera* (Hill ex Watson) Berk. & Broome, which is also sold and named as "cicada flower" in some areas in southern China.

Approximately 30 species of *Cordyceps* sensu lato and related genera are known to be pathogens of cicadas. Of these, *C. sobolifera*, *I. cicadae*, and *Cordyceps cicadae* (Miq.) Massee, are three New World names that have been widely used in the Old World (especially east Asia) without real reference, or comparison, to the type materials. Following the work of Sung & al. (2007), *C. sobolifera* was transferred to the genus *Ophiocordyceps* in the newly created family *Ophiocordycipitaceae*. The latter two taxa retained the same names within the family *Cordycipitaceae*. Historically, *Ophiocordyceps sobolifera* was the earliest named cordycipoid cicada pathogen (CCP), based on material from Guadeloupe and other islands of the Caribbean (Watson 1763). *Isaria cicadae* was later described by Miquel (1838) from a cicada in Brazil. The name *Cordyceps cicadae* was proposed for Miquel's taxon by Massee (1895) on the assumption that the *Isaria* was the 'imperfect' form of an unknown *Cordyceps*; however, no teleomorphic *Cordyceps* state has yet been described for a South American *I. cicadae*.

Throughout the twentieth century these three names were frequently applied to east Asian taxa of CCPs: with many collections from China, Japan, Korea, and Thailand, as well as New Zealand. Petch (1924, 1931, 1933, 1935, 1942) reviewed these names, as did Kobayasi (1939, 1941). Although Petch (1933) did not link *I. cicadae* and *C. sobolifera* in a list of synonyms, he later (Petch 1935, 1942) accepted *I. cicadae* as the anamorph of *C. sobolifera*. This link was also accepted by Mains (1958). Significantly, while Petch and Mains were not familiar with these fungi in the field, Kobayasi (1941) demonstrated that *I. cicadae* and *C. sobolifera* (as understood from Asian material) were unrelated.

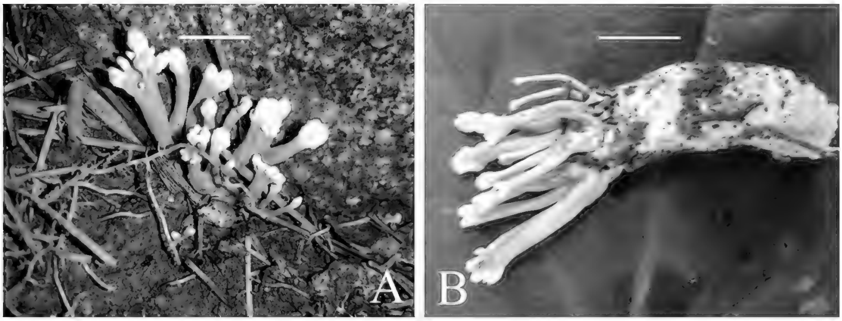

Fig. 1 Chan-hua (*Isaria cicadae*).
A: Emergent synnemata. B: Excavated Chan-hua. Scale bars = 10 mm.

In China, Shing (1975) recognised *C. sobolifera* and *I. cicadae* as distinct species based on Chinese collections of the two taxa although he accepted that the Asian *C. sobolifera* had an *Isaria* anamorph. Seemingly unaware of the Massee (1895) combination for the South American material, Shing (1975) further described a *Cordyceps* teleomorph that he associated with the Asian *I. cicadae*, and named this *Cordyceps cicadae* S.Z. Shing (an illegitimate later homonym of the Massee name). This anamorph–teleomorph link was based on the temporal and spatial co-occurrence of the two taxa in bamboo forest habitats in southern China and the proximity of the teleomorph specimens to collections of *I. cicadae* and to their being sold together in markets.

In 2012 and 2013, we collected specimens of CCPs from the Guniujiang Nature Preserve (Anhui Province, southeastern China). This is an area where medicinally valuable cicada 'cordyceps' have traditionally been collected. Our anamorph collections matched the Asian concept of *I. cicadae,* while our teleomorph specimens matched Shing's *C. cicadae*. However, isolates derived from ascospores produced an anamorph in culture that was not an *Isaria* (as had been concluded by Shing 1975). Rather, it belonged to the genus *Tolypocladium* based on morphological characteristics and a comparison of its 5.8S rDNA and ITS sequences. This *Tolypocladium* species is described here.

Materials & methods

Specimens and fungal isolates

Twenty-one specimens of CCPs were collected in bamboo forest from the Guniujiang Nature Preserve (Qimen County, southern Anhui, China, 30°01′N

117°31′E) at an elevation of approximately 420 metres above mean sea level. The specimens' hosts were soil-dwelling nymphs of an unidentified cicada. Isolates were derived from ascospores discharged onto glass slides according to the method of Li & al. (1999), RCEF6201 from GNJ130616-01 (= ZBAH632) and RCEF6202 from GNJ130617-02 (= ZBAH633). Many specimens of *I. cicadae* were also collected and isolated. One isolate of *I. cicadae* (RCEF0817-13) was further used in this study.

All specimens were freeze-dried and stored at 4°C after isolation and description as preparation for DNA extraction. The morphological characteristics of cultures incubated on potato dextrose agar (PDA) and Czapek-Dox agar at 25°C for 7 and 14 days were recorded. Specimen vouchers were conserved in the herbarium of Zhejiang BioAsia Pharmaceutical Co, Ltd., Zhejiang, China (ZBAH); and strains were conserved in the culture collection of Research Center on Entomogenous Fungi, Hefei, China (RCEF).

DNA extraction, PCR, and sequencing

Fresh mycelium (RCEF6201 and RCEF0817-13) was obtained from the surface of PDA plates that had been incubated at 25°C for 14 days. Approximately 0.1 g of sample was ground under liquid nitrogen in 1.5 ml Eppendorf tubes, and genomic DNA extracted using benzyl chloride according to a modification of the method described by Zhu & al. (1994).

The PCR mixtures, totalling 25 μL, were composed of 2.5 μL of 10 × Buffer (10 mM Tris/HCI, pH 8.3), 0.5 μL of a mixture containing 10 mM each of the four deoxyribonucleotide triphosphates (dNTP), 1.0 μL of each primer at 10 μmol/L (ITS4: 5′-TCCTCCGCTTATTGATATGC-3′ and ITS5: 5′-GGAAGTAAAAGTCGTAACAAGG-3′, Sango Biotech Co., Ltd., Shanghai), 0.2 μL of 5 U/μL DreamTaq polymerase (Fermentas, Thermo Scientific), 1 μL of template DNA, and 18.8 μL of double-distilled water.

The PCR was performed under the following temperature profile: 95°C for 5 min, followed by 37 cycles of 95°C for 30 s, 56°C for 30 s, and 72°C for 50 s, and a final extension at 72°C for 10 min. The PCR products were detected on an ethidium bromide (EB) gel (1.0% agarose gel including 0.5 g/ml EB).

The purification of the resulting products, as well as the sequencing of forward and reverse strands, was performed at the Beijing Genomics Institute (BGI). The sequencing data for the Anhui CCP (teleomorph and anamorph) and *I. cicadae* were submitted to GenBank.

Alignments and analysis

ITS1-5.8S-ITS2 sequences and those that we downloaded from GenBank were imported into the programme BioEdit Sequence Alignment Editor Version 4.8.6., aligned using Clustal X 2.0 for multiple sequence alignment and corrected manually (Hall 1999). The phylogenetic tree was constructed by the Neighbor-joining method (NJ) of MEGA 4.0 with 1000 bootstrap replicates. *Beauveria bassiana* (AF347611) was used as an outgroup taxon in the analyses. Bootstrap support of ≥70% was superimposed on all tree constructions.

Results

Molecular results

The ITS1-5.8S-ITS2 region of *I. cicadae* (GenBank KF740422) comprised 498 bp and showed a similarity of only 75.6% with the Anhui CCP. Both PCR products of the field-collected teleomorph and the culture of the Anhui CCP were detected by agarose gel electrophoresis. The amplified fragments spanning the ITS1-5.8S-ITS2 of the teleomorphic field material (GenBank KF696557) and the anamorphic culture (GenBank KF696558) were the same size (485 bp). The sizes of ITS1 (165 bp), 5.8S rDNA (146 bp), and ITS2 (174 bp) regions were also identical. This evidence confirmed that the isolate RCEF6201 was the anamorph of the field material ZBAH632, and not the *Isaria* anamorph of *C. cicadae* as concluded by Shing (1975).

To determine the phylogenetic position of the Anhui CCP, the sequences of the field material and the ex-ascospore strain RCEF6201 were compared with available sequences in GenBank via a BLAST search. These sequences revealed the Anhui CCP to be linked with the genus *Tolypocladium* (Fig. 2) and not with *Cordyceps cicadae* / *Isaria cicadae* as described by Shing (1975). However, the sequences and morphologies of the Anhui CCP and its cultured anamorph differed from those of other *Tolypocladium* spp.

Our study of the Anhui CCP revealed a new clade and suggested that it is a distinct taxon in *Tolypocladium* based on the molecular and morphological information. The teleomorph and culture of the CCP clustered in one group strongly supported by bootstrap proportions (BP = 100%). A sister-group relationship between *Tolypocladium paradoxum* (Kobayasi) C.A. Quandt & al. and the Anhui CCP was also strongly supported (BP = 100%) (Fig. 2).

BLAST searches in GenBank suggested that there are several *Tolypocladium* species similar to the Anhui CCP. The Anhui CCP shared 98.1% homology with *T. paradoxum* (AB027369), which is also pathogenic on cicada nymphs, and 94.9% with *Tolypocladium ophioglossoides* (J.F. Gmel.) C.A. Quandt & al. (AJ309360), which is pathogenic on *Elaphomyces*. It also shared a high level of ITS sequence identity with a sequence from China identified as *Cordyceps imagamiana* Kobayasi & Shimizu. Although there was little molecular divergence between the Anhui CCP and *C. imagamiana*, the morphological differences between the Anhui CCP and the original Japanese description of *C. imagamiana* were noticeable (Kobayasi & Shimizu 1983, Shimizu 1997 Plate 23). On the basis of these results we describe the Anhui CCP as a new species of *Tolypocladium* (sensu Quandt & al. 2014) in accordance with the one fungus = one name (1F = 1N) principle.

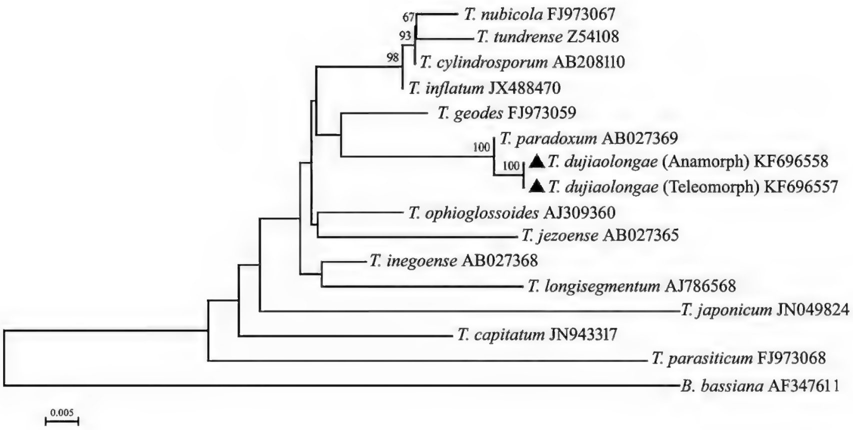

Fig. 2 Neighbor-joining tree based on ITS1-5.8S-ITS2 region sequence data from *Tolypocladium dujiaolongae* and related species. Values above the branches indicate bootstrap support.

Taxonomy

Tolypocladium dujiaolongae Y.P. Cao & C.R. Li, **sp. nov.** Figs 3, 4

MycoBank MB821894

Differs from *Cordyceps cicadae* S.Z. Shing by its larger perithecia and larger asci; and from *C. imagamiana*, *Tolypocladium inegoense*, and *T. paradoxum* by its blackish-brown to blackish-purple stipe, its brown to black fertile parts, its larger perithecia, and stipe with or without rhizoids attached to the host.

Type: China. Anhui Province: Qimen County, National Natural Reserve of Guniujiang, 30°01′N 117°31′E, alt. 420 m, 16.VI.2013, Y.P. Cao GNJ130616-01 (**Holotype**, ZBAH632; GenBank KF696557; ex-ascospore culture, RCEF6201, GenBank KF696558).

Etymology: *dujiaolongae* refers to the traditional Chinese name for the species, which translates as "single-horned dragon".

Stroma arising from the head of the nymphal cicada host, blackish brown to blackish-purple, fleshy, mostly solitary, occasionally branched, erect or curving, 26–70 (rarely 105) mm long, clavate or obclavate, corniform or oblate, with a blunt or slightly apiculate and tuberculate tip and sunken grooves. Stipe cylindric, 5–10 mm thick, 10–45 mm long, brown or yellow. Fertile part clavate, 40–55 × 4–12 mm, clearly defined from the stipe, punctate with perithecial ostioles, brown to black, without sterile tip. Perithecia ampullaceous, wholly immersed, (233–)520–740(–780) × (250–)300–330

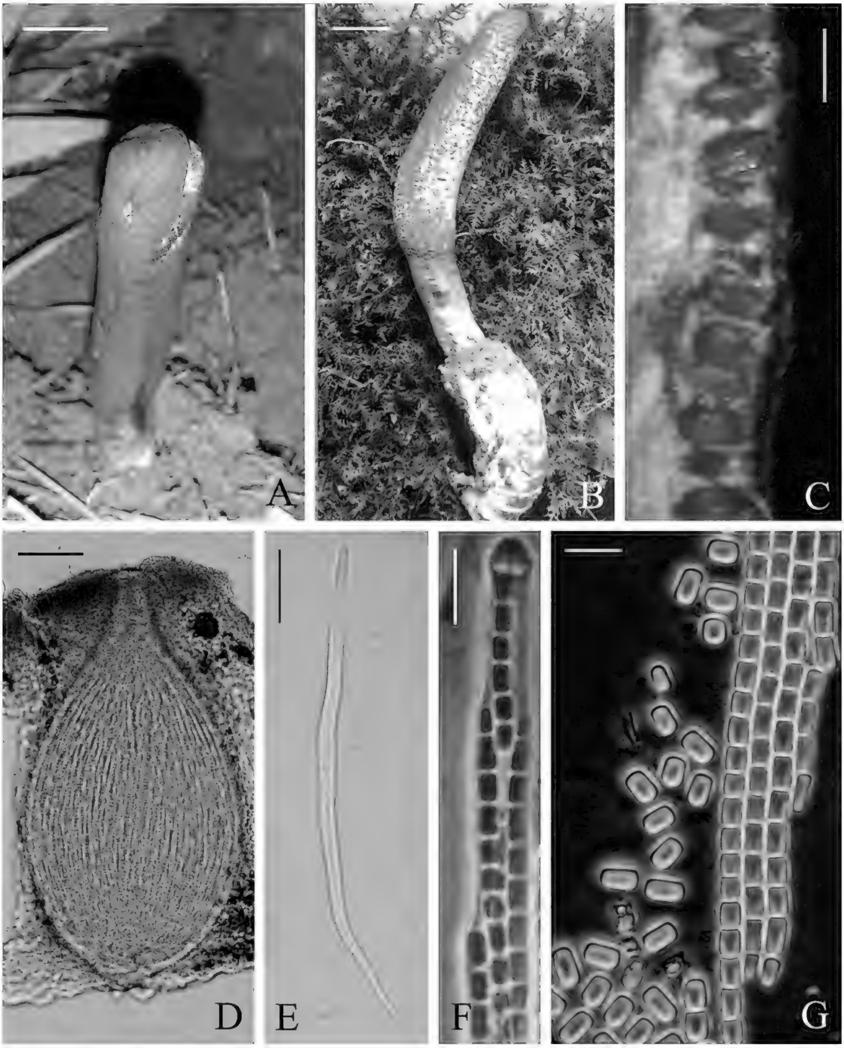

Fig. 3 *Tolypocladium dujiaolongae* teleomorph. A, B: Stroma of *Tolypocladium dujiaolongae*; C: Wholly immersed perithecia; D: Perithecium; E: Whole ascus; F: Upper part of ascus; G: Part spores. Scale bars: A, B = 10 mm; C = 500 µm; D = 100 µm; E = 50 µm; F, G = 10 µm.

(–360) µm, with wall 23.0–33.5 µm thick and ostioles 34–80 µm in diameter. Asci 8-spored, hyaline, cylindrical, (380–)420–468(–500) × 8–11 µm. Ascus cap hemispherical, 5.5–9.0 µm in diameter, 4.0–8.0 µm high. ascospores hyaline, filiform, 240–310 µm long when discharged, smooth, multiseptate,

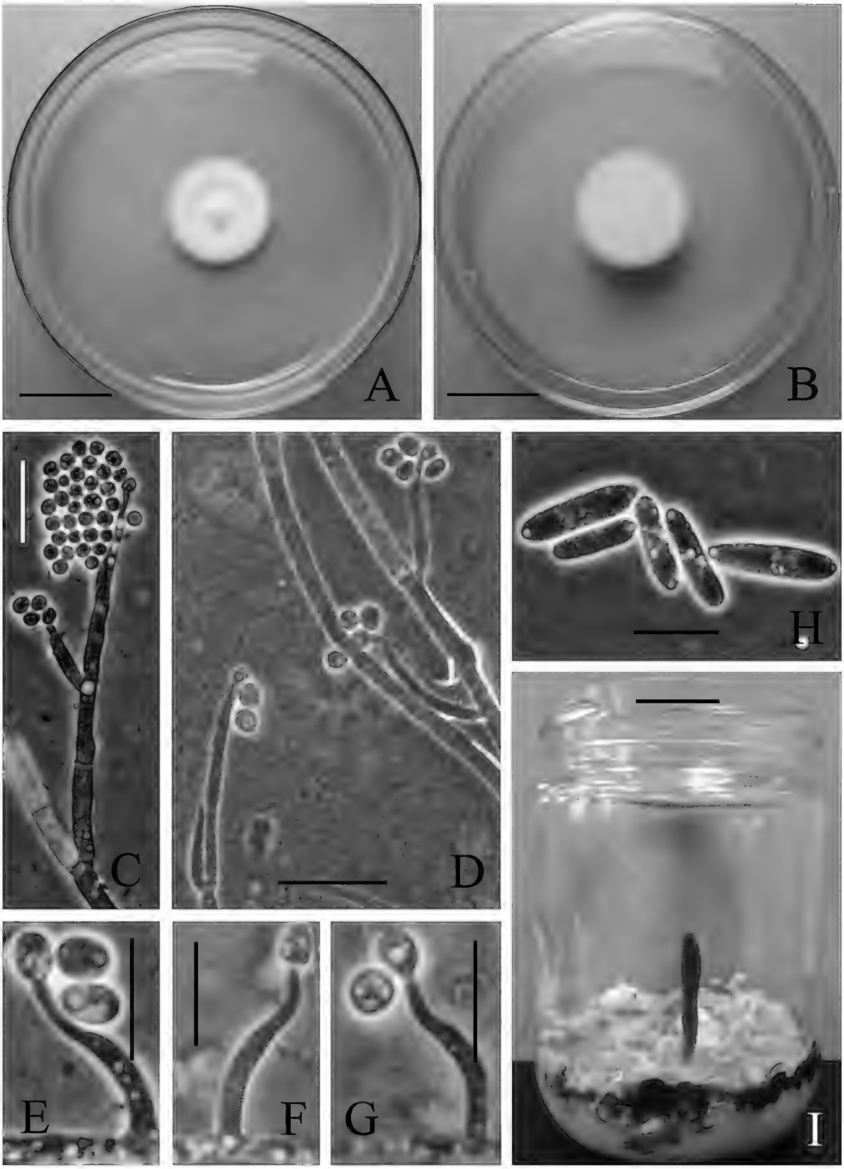

Fig. 4 *Tolypocladium dujiaolongae* anamorph. A: Colony on PDA (25°C, 14 d); B: Colony, reverse side; C–H: Conidiophores and conidia; I: Stroma of *Tolypocladium dujiaolongae* on artificial media. Scale bars: A, B = 2 cm; C–H = 10 µm; I = 20 mm.

breaking into cylindrical part spores, 3–5(–7.0) × 2–3 µm, germinating at 25°C on PDA.

Colonies growing slowly, 10–15 mm in diameter after 7 days on Czapek-Dox agar at 25°C; velutinous, loose, white to yellowish, hemispherical with a 2–3 mm high region protruding in the colony centre. Reverse white. Colonies on PDA growing moderately at 25°C after 7 days, 16.0–18.0 mm diam., and 28–30 mm after 14 days on PDA, velutinous, loose, white. Reverse centre yellow-orange to dark brownish, with white margin. Hyphae septate, smooth-walled, hyaline, 2.0–3.5 µm wide. Phialides solitary, occasionally verticillate, (5–)11–35(-52) × 1.0–2.7 µm, usually growing on the aerial mycelium or on simple conidiophores, cylindrical or conical, slightly swollen basally, narrowly tapering into a distinct neck approximately 1.0 µm wide, terminal phialides generally elongate; neck often bent without clear denticulate scars. Conidia one-celled, hyaline, smooth-walled, globose to ovoid when separate, and polyhedral when aggregated, (2.5-)3–4(–4.7) × 2.4–3.7 µm, aggregating mostly in small heads, 11.0–36.8 µm in diameter and occasionally in chains at the tips of the phialides; a very small proportion of the conidia much larger, long ellipsoidal, approximately 5–9 µm wide and up to 8–25(–36) µm long.

Ecology & distribution—Scattered in bamboo forest. Summer. Common in southern China, especially in Zhejiang, Anhui, Jiangsu, Jiangxi, and Fujian provinces.

Additional specimen examined — China. Anhui Province: Qimen County, National Natural Reserve of Guniujiang, 30°01′N 117°31′E, alt. 420 m, 16.VI.2013, Y.P. Cao GNJ130617-02 (ZBAH633; ex-ascospore culture RCEF6202).

Discussion

Taxonomy and phylogeny

The macroscopic and microscopic characteristics of *T. dujiaolongae* specimens collected from the Guniujiang Nature Preserve were nearly identical to those described as *C. cicadae* by Shing (1975), especially with regards to the shape and size of the part spores but with exceptions for the larger perithecia and longer asci (Table 1). The teleomorphs of *T. paradoxum*, *Tolypocladium inegoense* (Kobayasi) C.A. Quandt & al., and *C. imagamiana* were compared with *Tolypocladium dujiaolongae* (Table 1, Fig. 2). These are all parasitic on cicada nymphs and produce cylindrical part spores. Although the ITS sequences were similar there are obvious differences between *T. paradoxum* and *T. dujiaolongae* in the appearance of their stromata. Those of *T. paradoxum* are pale purple and attached to the host by subterranean whitish rhizoids

(Shimizu 1997, Plate 24). In comparison, the stroma of *T. dujiaolongae* is brown or yellow and simple attached directly to the host without rhizoids.

Tolypocladium inegoense is easily distinguished from *T. dujiaolongae* by the superficial (but crowded) nature of its perithecia compared with the immersed form of *T. dujiaolongae* and the colour of its fertile part which is a dark olive green compared with the blackish brown of *T. dujiaolongae* (Shimizu 1997 Plate 14). According to the description and the picture of *C. imagamiana* (Kobayasi & Shimizu 1983; Shimizu 1997 Plate 23), *T. dujiaolongae* and *C. imagamiana* are also quite different in the appearance of their stromata. The latter have longer stromata (90 mm) and a thinner stipe (1.5–2.0 mm) with a pallid ochre and oblong fertile part. We have no further information regarding the specimen identified as *C. imagamiana* from China and consider this named to have been misapplied.

The morphological features of the cultured anamorph of *T. dujiaolongae* are also consistent with the genus *Tolypocladium*. Four species in this genus (*T. inflatum* W. Gams, *T. cylindrosporum* W. Gams, *T. geodes* W. Gams, and *T. tundrense* Bissett) are similar to the anamorph of *T. dujiaolongae*. The conidiogenous cells of *T. dujiaolongae* are similar in shape and size to those of *T. geodes*, but this species differs in only having one type of conidia, either subglobose or broadly obovoid, and some of its strains on Czapek-Dox's agar are dark green or bluish (Gams 1971). *Tolypocladium inflatum* and *T. cylindrosporum* not only have one type of conidia like *T. geodes*, but the conidiogenous cells of both species are ellipsoidal to subglobose at the lower part. In contrast, those of the cultured anamorph of *T. dujiaolongae* are cylindrical or only slightly swollen basally (Gams 1971). *Tolypocladium tundrense* resembles the anamorph of *T. dujiaolongae* in producing two types of conidia, but their conidial size and shape are different (Bissett 1983). Furthermore, these four species exhibit solitary and verticillate phialides (Table 1).

Gams (1971) established the genus *Tolypocladium* with the type species *T. inflatum*, and described three soil-inhabiting species, *T. inflatum*, *T. cylindrosporum* and *T. geodes*. Since then many species have been described in the genus. However, most *Tolypocladium* species have been described from soil samples and as a result their true role in the soil remains largely unknown. Although well known as saprobes commonly found in the soil, many *Tolypocladium* species are also entomopathogenic fungal parasites on a range of insects, rotifers, nematodes, and other fungi. Hodge & al. (1996) first established the relationship between *Tolypocladium* and *Cordyceps* sensu

TABLE 1. Morphological comparison of *Tolypocladium dujiaolongae* with allied *Cordyceps* and *Tolypocladium* species

SPECIES	CHARACTER				
	STIPE COLOUR	FERTILE PART	PERITHECIUM	ASCUS	ASCUS CAP (diam)
C. cicadae		Black brown	Perpendicularly immersed; 350–540 × 115–300 µm	262.5–378 × 6.2–9.1 µm	
T. paradoxum	Pale purple, whitish mycelial strands onto host	Dark purple	Wholly immersed; 480–500 × 300–350 µm	9–10 µm thick	4 µm
T. inegoense	White	Dark olive green	Superficial: 520–550 × 260–280 µm	400–450 × 7–7.5 µm	5–6.5 µm
C. imagamiana	White	Pallid ochre, oblong	Immersed 450–550 × 250–275 µm	7 µm thick	4 µm
T. dujiaolongae	Brown or yellow	Brown or black	Wholly immersed 600–780 × 270–360 µm	380–500 × 8–11 µm	5.5–6.5 µm

lato demonstrating that *Cordyceps subsessilis* Petch has a *Tolypocladium* anamorph.

Sung & al. (2007) recognised *Tolypocladium* and *Tolypocladium*-producing teleomorphs in the newly erected family *Ophiocordycipitaceae* (*Hypocreales*). Species were placed in the "C. ophioglossoides clade" and a new genus, *Elaphocordyceps* G.H. Sung & Spatafora, was erected to accommodate these taxa (Sung & al. 2007). However, following the 2011 Nomenclature Session of the XVIII International Botanical Congress in Melbourne, a single system of nomenclature, or "one fungus = one name" (1F = 1N), became effective 1 January 2013, regardless of life history states. In order to reflect changes in Article 59 of the ICN, Quandt & al. (2014) emended the family *Ophiocordycipitaceae* based on molecular phylogenetic analyses and proposed six genera encompassing *Drechmeria*, *Harposporium*, *Ophiocordyceps*, *Polycephalomyces*, *Purpureocillium*, and *Tolypocladium*; *Elaphocordyceps* is no longer a valid name in the 1F = 1N framework, and species were transferred to the genus *Tolypocladium*.

As with *I. cicadae* it would appear that *T. dujiaolongae* has probably also had a long history of usage in TCM under the general name of 'cicada flower'. We have found no documented records of human consumption of

T. dujiaolongae causing problems. Studies on the effects of artificial culture conditions on the artificial fruit body formation of *T. dujiaolongae*, submerged fermentation of strains, and detection of myriocin and other metabolites are ongoing by our team.

Acknowledgments

The authors thank Drs G.H. Sung, C.L. Hou, and B. Shrestha for reviewing and improving the manuscript. We especially thank the late Dr Walter Gams for his discussions on adopting the word "cordycipoid" to describe taxa in *Clavicipitaceae, Cordycipitaceae,* and *Ophiocordycipitaceae.* This work was supported by the National High Technology Research and Development Program of China (863 Program, No. 2007AA021506) and the National Natural Science Foundation of China (No. 30570004).

Literature cited

Bissett J. 1983. Notes on *Tolypocladium* and related genera. Canadian Journal of Botany 61: 1311–1329. https://doi.org/10.1139/b83-139

Gams W. 1971. *Tolypocladium*, eine Hyphomycetengattung mit geschwollenen Phialiden. Persoonia 6: 185–191.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Hodge KT, Krasnoff SB, Humber RA. 1996. *Tolypocladium inflatum* is the anamorph of *Cordyceps subsessilis.* Mycologia 88: 715–719. https://doi.org/10.2307/3760965

Kobayasi Y. 1939. On the genus *Cordyceps* and its allies on cicadae from Japan. Bulletin of the Biogeographical Society of Japan 9: 145–176.

Kobayasi Y. 1941. The genus *Cordyceps* and its allies. Science Reports of the Tokyo Bunrika Daigaku, Section B, 5: 53–260.

Kobayasi Y, Shimizu D. 1983. *Cordyceps* species from Japan 6. Bulletin of the National Science Museum, Tokyo, series B, 9: 1–21.

Li ZZ, Li CR, Huang B, Fan MZ, Lee MW. 1999. New variety of *Cordyceps gunnii* (Berk.) Berk. and its *Paecilomyces* anamorph. Korean Journal of Mycology 27: 231–233.

Mains E. 1958. North American entomogenous species of *Cordyceps*. Mycologia 50: 169–222. https://doi.org/10.2307/3756193

Massee G. 1895. A revision of the genus *Cordyceps*. Annals of Botany 9: 1–44. https://doi.org/10.1093/oxfordjournals.aob.a090724

Miquel FAW. 1838. Sur une espèce nouvelle d'*Isaria*, du Brésil. Bulletin des Sciences Physiques et Naturelles en Néerlande 36: 85–86.

Petch T. 1924. Studies in entomogenous fungi: IV. Some Ceylon *Cordyceps*. Transactions of the British Mycological Society 10: 28–45. https://doi.org/10.1016/S0007- 1536(24)80005-0

Petch T. 1931. Notes on entomogenous fungi. Transactions of the British Mycological Society 16: 55–75. https://doi.org/10.1016/S0007-1536(31)80006-3

Petch T. 1933. Notes on entomogenous fungi. Transactions of the British Mycological Society 18: 48–75. https://doi.org/10.1016/S0007-1536(33)80026-X

Petch T. 1935. Notes on entomogenous fungi. Transactions of the British Mycological Society 19: 161–194. https://doi.org/10.1016/S0007-1536(35)80008-9

Petch T. 1942. Notes on entomogenous fungi. Transactions of the British Mycological Society 25: 250–265. https://doi.org/10.1016/S0007-1536(42)80017-0

Quandt CA, Kepler RM, Gams W, Araújo JPM, Ban S, Evans HC, Hughes D, Humber R, Hywel-Jones N, Li Z, Luangsa-ard JJ, Rehner SA, Sanjuan T, Sato H, Shrestha B, Sung G-H, Yao Y-J, Zare R, Spatafora JW. 2014. Phylogenetic-based nomenclatural proposals for *Ophiocordycipitaceae* (*Hypocreales*) with new combinations in *Tolypocladium*. IMA Fungus 5: 121–134. https://doi.org/10.5598/imafungus.2014.05.01.12

Shimizu D. 1997. Illustrated Vegetable Wasps and Plant Worms in Colour. Tokyo, Ie-no-hikari Association.

Shing SZ. 1975. Classification of *Cordyceps sobolifera* (Hill) Berk. et Br. and *Cordyceps cicadae* Shing sp. nov. (in Chinese). Acta Microbiologica Sinica 15: 21–26.

Sung GH, Hywel-Jones NL, Sung JM, Luangsa-ard JJ, Shrestha B, Spatafora JW. 2007. Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. Studies in Mycology 57: 5–59. https://doi.org/10.3114/sim.2007.57.01

Watson W. 1763. An account of the insect called the Vegetable Fly. Philosophical Transactions of the Royal Society 53: 271–274. https://doi.org/10.1098/rstl.1763.0045

Zhu H, Qu F, Zhu LH. 1994. Isolation of genomic DNAs from fungi using benzyl chloride (in Chinese). Acta Mycologica Sinica 13: 34–40.